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Citation for published version:
VERVELDE, L, VERMEULEN, AN & JEURISSEN, SHM 1993, 'COMMON EPITOPES ON EIMERIA-TENELLA SPOROZOITES AND CECAL EPITHELIUM OF CHICKENS', *Infection and Immunity*, vol. 61, no. 10, pp. 4504-4506.

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Document Version:
Publisher's PDF, also known as Version of record

Published In:
Infection and Immunity

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Infection and Immunity

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L Vervelde, A N Vermeulen and S H Jeurissen
Infect. Immun. **1993, 61(10):4504.**

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INFECTION AND IMMUNITY, Oct. 1993, p. 4504–4506
0019-9567/93/104504-03$02.00/0
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Vol. 61, No. 10

Common Epitopes on *Eimeria tenella* Sporozoites and Cecal Epithelium of Chickens

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Received 26 May 1993/Accepted 2 July 1993

***Eimeria tenella* sporozoites invade the intestinal epithelium at a highly specific site. Certain molecules that were recognized by monoclonal antibody E.TEN 11M-2, which was prepared against *E. tenella* sporozoites, were detected on both cecal epithelium and sporozoites. We expect that these molecules are involved in attracting sporozoites towards the site of entry.**

Eimeria species not only are host specific but parasitize specific sites within a host. In chickens, *Eimeria* species parasitize different regions of the intestine. For example, *Eimeria acervulina* penetrates the upper small intestine while *E. tenella* invades the cecum. Although Joyner (4) suggested that the interval between infection with oocysts and the release of sporozoites may be related to site specificity, it now appears that certain properties of the invasion site may determine site specificity. Support for the latter hypothesis was provided by studies in which sporozoites were injected intravenously, intramuscularly, and intraperitoneally, resulting in infections in the same area of the intestine, as would be expected if the parasites had been administered via the natural route (4, 6). This note describes the detection of molecules both on the cecal epithelium and on sporozoites by a single monoclonal antibody (E.TEN 11M-2) prepared against *E. tenella* sporozoites. Monoclonal antibody E.TEN 11M-2 was obtained through injection of BALB/c mice with *E. tenella* sporozoites. The spleen cells were fused with a myeloma cell line (P3X63AG 8.6.5.3) and cloned as described by Schönherr and Roelofs (7). The sporozoites were released in vitro from hypochlorite-sterilized sporulated oocysts (5). Figure 1 shows the reaction of E.TEN 11M-2 on air-dried, acetone-fixed *E. tenella* sporozoites. Apparently, the target protein was located on the surface of the sporozoite. This was confirmed by using live intact sporozoites as an antigen (data not shown). In immunoblotting after nonreducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the monoclonal antibody recognized an 18- to 19-kDa protein (data not shown).

The monoclonal antibody was tested for its in vivo staining pattern by using White Leghorn (W.L.A.) chickens which were specific pathogen free and were 1 day, 2 weeks, and 8 weeks old. The chickens were exsanguinated, and various parts of the digestive tract (i.e., the esophagus, proventriculus, duodenum, jejunum, cecum, and colon) and all other tissues used in this study were removed, snap-frozen in liquid nitrogen, and stored at −20°C. Cryostat sections of chicken tissue were examined with immunoperoxidase staining techniques as described elsewhere (3).

The most striking feature of E.TEN 11M-2 was the site-specific reaction with the cecal epithelium. E.TEN 11M-2 specifically recognized granula, localized at the apical region, in columnar epithelial cells in the cecum. Staining was most intense at the tops of the villi and was evident throughout the villus but was scarcely visible in basal crypt cells (Fig. 2). Isolation of epithelial cells with Ca^{2+}-Mg^{2+}-free medium and 2 mM EDTA was followed by Percoll-based separation of rounded young cells and older columnar cells. The cells were air dried on slides, acetone fixed, and incubated with monoclonal antibody E.TEN 11M-2. Figure 3 shows the specific reaction with the granular material in the isolated cells and the granula remaining after cell lysis.

The in vivo staining pattern revealed that the epithelium above the cecal tonsil stained slightly but the rest of the cecal epithelium stained vividly. The striking gradual staining pattern within the villus coincides exactly with the site where the sporozoites invade the cecum. The epithelium in other parts of the digestive tract (i.e., the esophagus, proventriculus, duodenum, and jejunum) showed no staining. Only the proximal part of the colon showed slight staining of the columnar epithelium. This result is very striking, because this part can also be invaded by *E. tenella* during heavy infections. To determine whether the molecule is constitutively expressed by the cecal epithelium, the ceca of chicken embryos at days 12, 15, and 19 of incubation were examined. Staining of the epithelium was first detected in

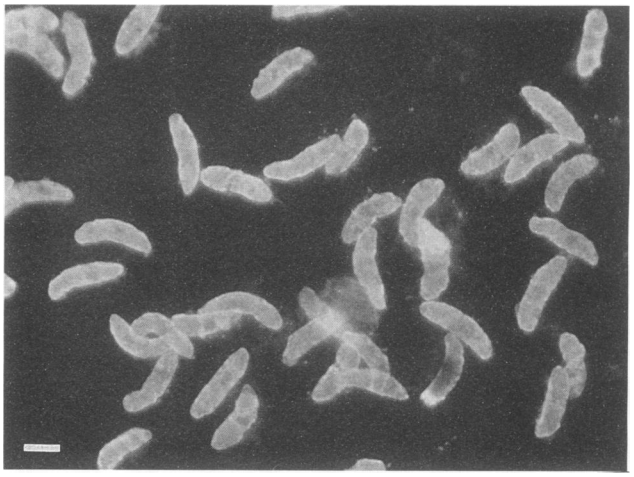

FIG. 1. Immunofluorescence pattern of E.TEN 11M-2 on air-dried *E. tenella* sporozoites. Bar, 5 μm.

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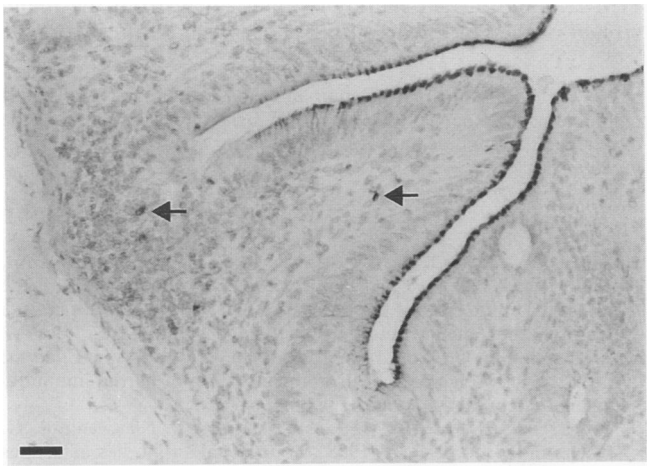

FIG. 2. Cryostat section of chicken cecum incubated with E.TEN 11M-2, peroxidase conjugate, and 3,3'-diaminobenzidine tetrahydrochloride to develop peroxidase activity. In addition to sporozoites (arrows), granula in the apical region of the epithelium were also vividly stained. Bar, 50 μm.

embryos at day 15 of incubation. Thus, the molecule is constitutively expressed by the cecal epithelium. Furthermore, the influence of infection on the expression of the molecule was examined. Therefore, 3-month-old chickens, either immunized with *E. tenella* or not, were exsanguinated and the duodenum, cecum, and colon were removed. The fact that E.TEN 11M-2 recognized molecules in naive chickens means that these molecules originated from the chickens themselves and not from the parasites during infection. When the ceca of repeatedly infected chickens were examined, the same specific staining of the epithelium was detected. Therefore, earlier infection with *E. tenella* did not diminish expression of the molecule.

Furthermore, we determined whether E.TEN 11M-2 reacted with sporozoites of other *Eimeria* species, by using immunofluorescence on air-dried antigens. However, E.TEN 11M-2 did not react with sporozoites of *E. acervulina* and *E. maxima*. Although various other monoclonal antibodies of the same isotype and an antiserum were used and all were specific for different antigens of sporozoites and other developmental stages of *E. tenella*, none of them recognized the cecal epithelium. Other sorts of epithelia, outside the gut, such as cubical kidney epithelium, pseudostratified ciliated columnar epithelium of the trachea, stratified squamous epithelium of the tongue, stratified squamous keratinizing epithelium of the skin, and lung epithelium, were also examined. However, none of these epithelia stained with E.TEN 11M-2. This result indicates that the cecal epithelium expresses a site-specific cellular determinant in common with *E. tenella* sporozoites. Furthermore, E.TEN 11M-2 was also compared with specific cell components of epithelia. E.TEN 11M-2 did not stain keratin, as was demonstrated by using a double-staining technique with rabbit antiserum against keratin (Dakopatts, Santa Barbara, Calif.) that was performed as described elsewhere (3). The E.TEN 11M-2 staining pattern also differed from that described for villin (8). Although chickens are the natural host of *E. tenella*, *E. tenella* also invades turkeys exclusively via the cecal epithelium (2). Therefore, E.TEN 11M-2 was tested on cryostat sections of duodenum, ileum, cecum, and colon tissues of 10-week-old British United Turkeys (BUT Big 6) to determine whether the molecule was a unique trait of the natural host. E.TEN 11M-2 did not react with the epithelium of the small intestines and the colon, but it did recognize identical epitopes on the cecal epithelia of turkeys. We conclude that E.TEN 11M-2 recognizes a molecule that is present both on sporozoites of *E. tenella* and on the cecal epithelia of both turkeys and chickens. This molecule, which does not resemble cytoskeletal elements, is constitutively expressed by the cecal epithelium. Expression of the molecule does not seem to be affected by *E. tenella*.

The staining pattern of monoclonal antibody E.TEN 11M-2 suggests a functional role of the recognized epitope on both sporozoite and host cells. Three types of interactions can be suggested.

(i) A zipper-like interaction between molecules on sporozoites and molecules on the epithelium might be possible, because an identical molecule was detected on both sporozoite and epithelial cells. However, in contrast to the site specificity that is exhibited in vivo, *E. tenella* can easily invade cell cultures prepared from kidneys of gallinaceous birds. As shown in the results, the E.TEN 11M-2 epitope is not present on kidney epithelial cells. This indicates that although the possibility of a zipper-like interaction in vivo cannot be excluded, other mechanisms can also establish the

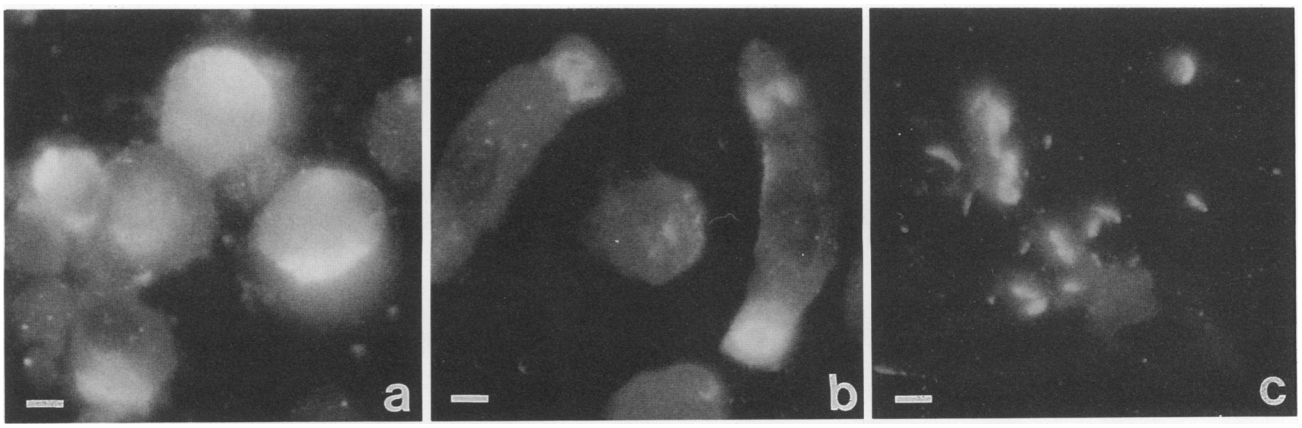

FIG. 3. Immunofluorescence pattern of E.TEN 11M-2 on isolated, noninfected cecal epithelial cells form 3-week-old specific-pathogen-free chickens. Panels: a, young, rounded cells; b, older, columnar cells; c, granula remaining after lysis of columnar cells. Bar, 5 μm.

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interaction by which sporozoites invade epithelial cells in vitro.

(ii) During evolution, *Eimeria* sporozoites may have developed antigenic mimicry to evade the host's immune responses. Acquisition of host molecules may protect the parasite from recognition. If the sporozoites can retard the host's immune response during invasion and migration to the crypts, then the parasite will increase its chance to develop into schizonts and continue its life cycle.

(iii) Epithelial components can well act as recognition molecules that originate uniquely from the various segments of the intestine and function as local signals for the parasites. In this study, identical epitopes were recognized by E.TEN 11M-2 on the cecal epithelia of chickens and turkeys. This suggests that the site specificity of the parasite is determined by characteristics of intestinal cells that are shared by a number of hosts rather than by a unique trait of the natural host. This hypothesis is strengthened by the fact that in vivo sporozoites invade the same intestinal sites whether in chickens, the natural host, or in turkeys, in which the parasite does not complete its cycle (2). The uniqueness of the various segments of the chicken intestine is also reflected in the fact that the composition of carbohydrate-rich macromolecules in the intestinal epithelium varies. Alroy et al. (1) reported that *Lens culinaris* agglutinin has a high affinity for the luminal surface of the cecal epithelium. It has a moderate affinity for the corresponding sites in colonic epithelium but has no affinity for the small intestine. The differences in sugar specificity between these lectins thus reflect the differences in the presence of carbohydrate residues at different sites along the intestine. These carbohydrate residues may function as chemotactic recognition molecules in the intestinal lumen because epithelial cells in the intestine are continuously being shed. We expect that a positive gradient of epithelial components in the lumen, recognized by E.TEN 11M-2, could attract the sporozoites away from the mainstream of the gut towards its site of invasion, the cecum.

We thank Corinne van Geffen and Jos Panhuijzen for expert technical assistance, F. van Meel and his group for preparing the monoclonal antibodies, and Herman Peek for the generous gift of *E. maxima* oocysts.

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